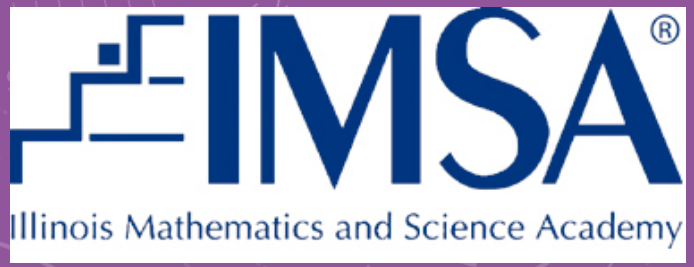


THE DEVELOPMENT OF A HUMANIZED ANTIBODY-TARGETED ABO-SPECIFIC PET PROBE FOR EARLY DIAGNOSTIC IMAGING OF ALZHEIMER'S DISEASE

Adrian Bebenek
Mentors: Ms. Kirsten Viola and Dr. William Klein

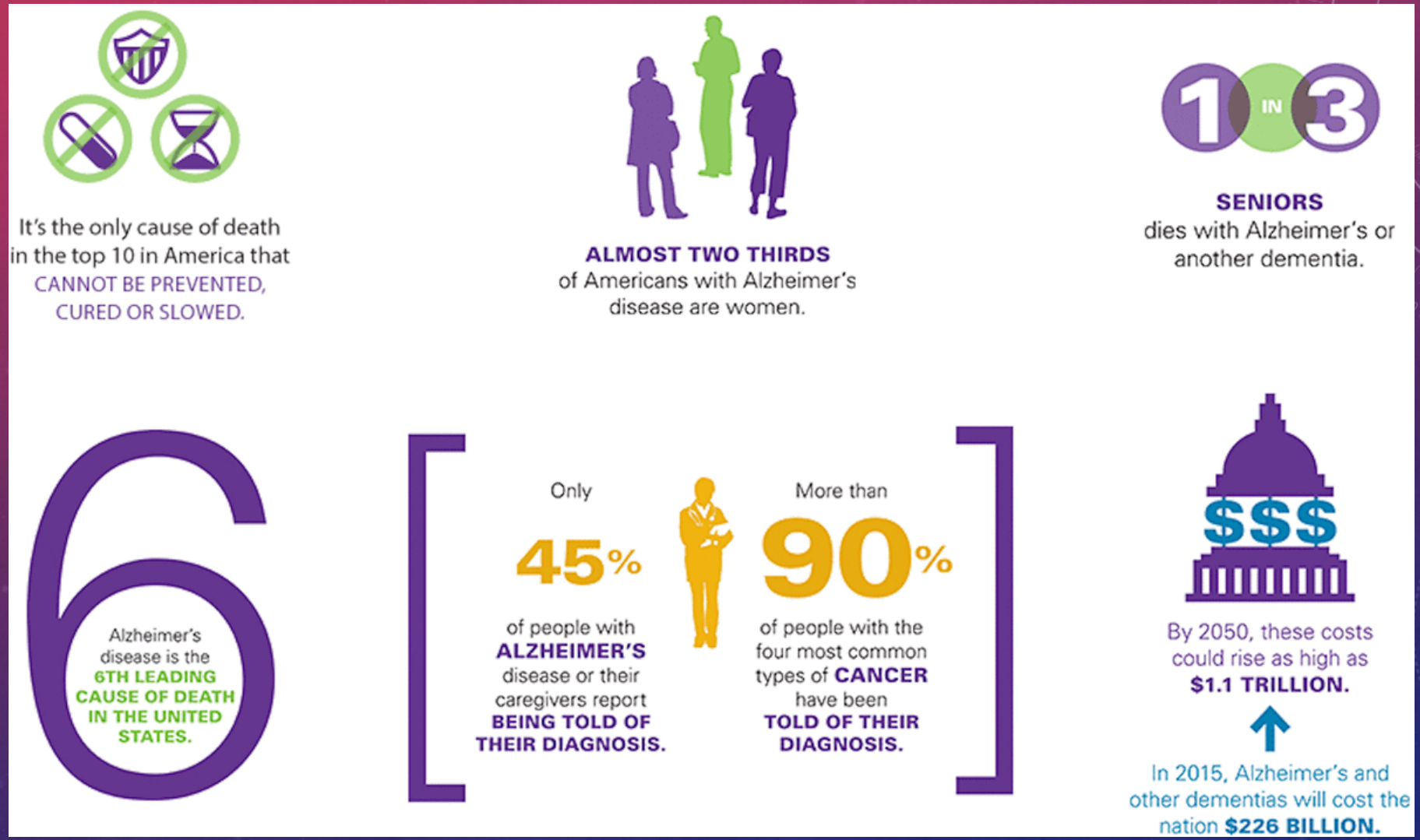
Kirsten L. Viola[1], Adrian Bebenek[1], Jake Vitrofsky[1], Maira Bicca[2], Erika N. Cline[1], Alex L. Qin[1], Chad Haney[1], Ting-Tung Chang[3], and William Klein[1, 4]

[1] Northwestern University
[2] Universidade Federal de Santa Catarina
[3] Rethink Imaging
[4] Acumen Pharmaceuticals

TOPICS

- Topic 1 – General Background
- Topic 2 – Molecular Basis
- Topic 3 – Design of PET Probe
- Topic 4 – Preservation of Immunoreactivity
- Topic 5 – NU4PET Preliminary Injections
- Topic 6 – NU4 Histology and Morphology
- Topic 7 – ACU193 Histology and Morphology
- Topic 8 – ACU193PET Preliminary Injections

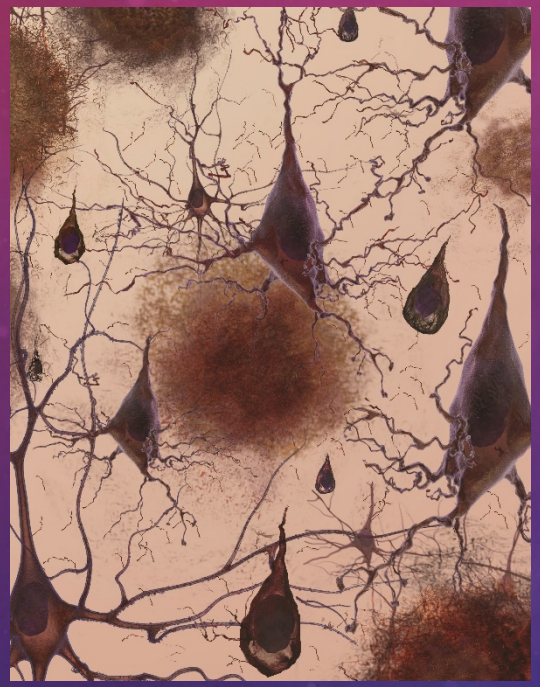
BACKGROUND



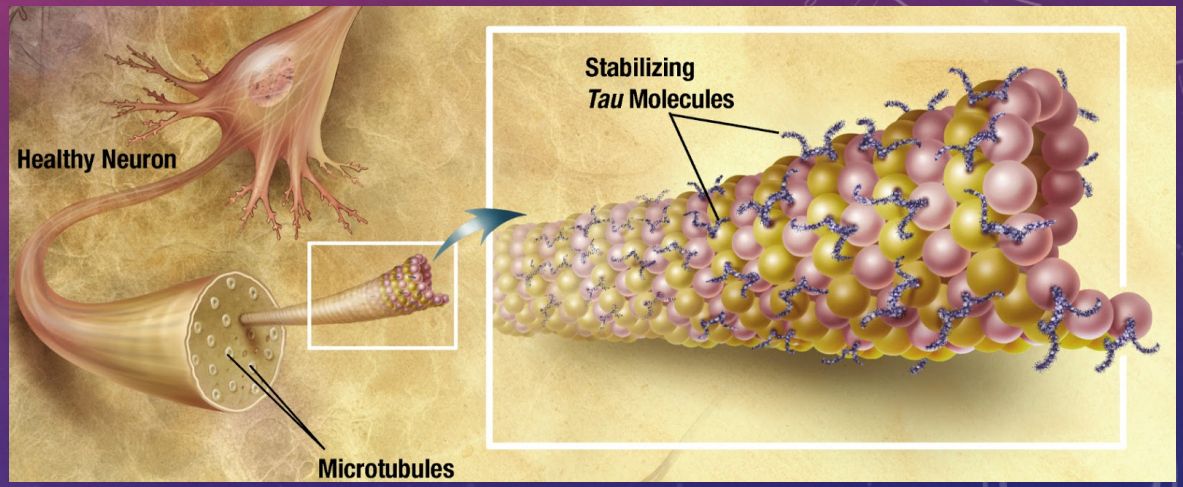
Alzheimers Association 2015

MOLECULAR BASIS

- Amyloid-β plaques
- Neurofibrillary tangles



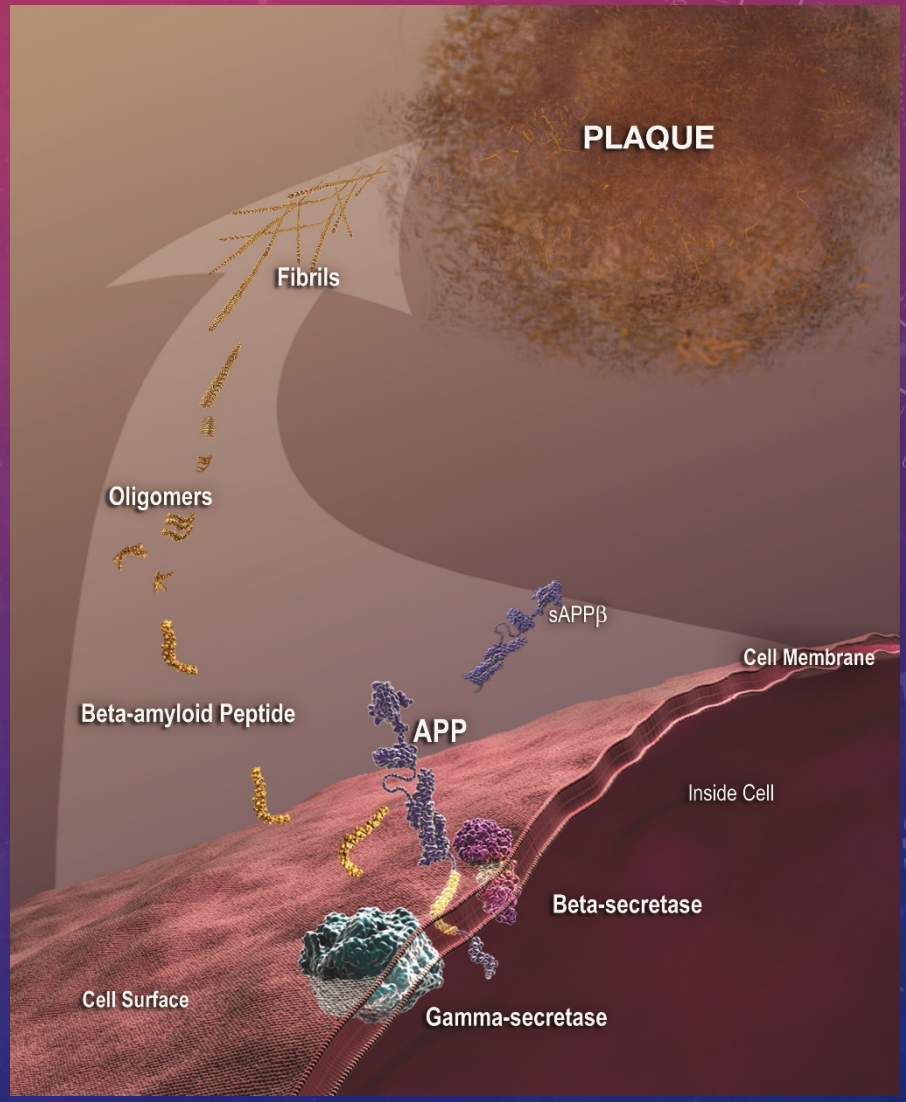
PBS 2016



Lieff 2015

AMYLOID BETA PLAQUES

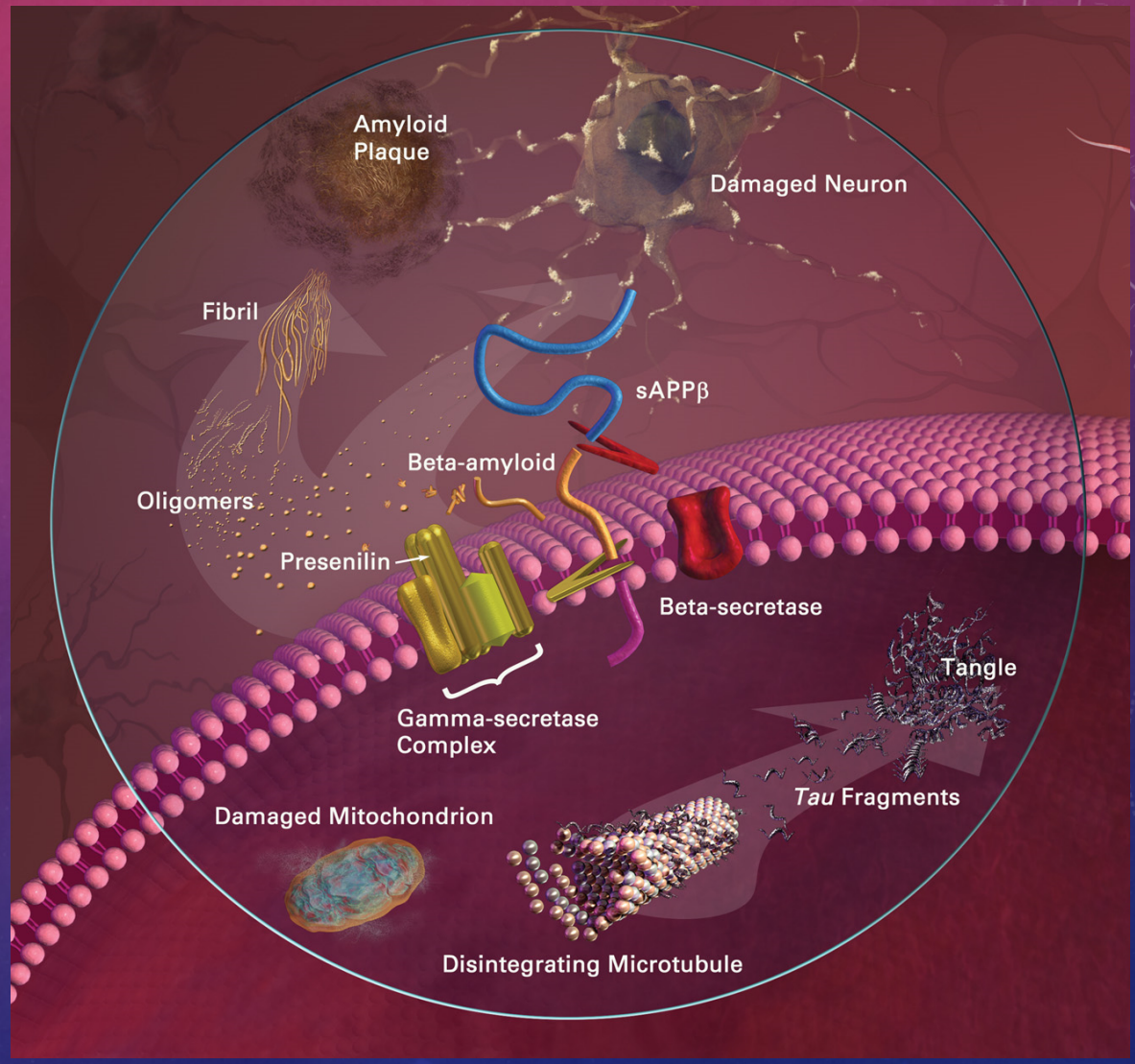
- Amyloid Precursor Protein (APP)
- $A\beta_{42}$ vs $A\beta_{40}$
- Peptide → oligomer → fibril → plaque



NIA 2016

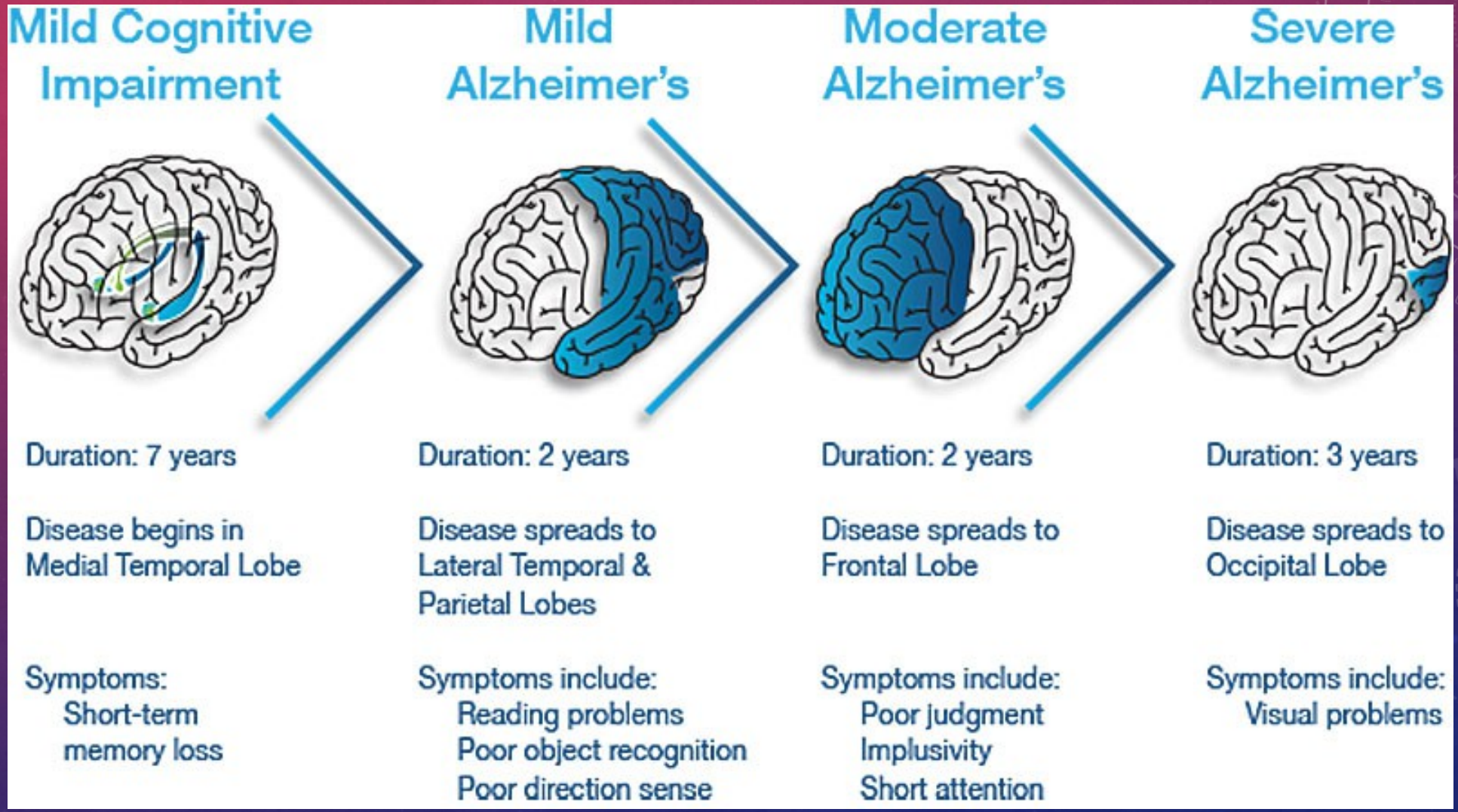
AMYLOID CASCADE HYPOTHESIS

- Weak correlation between AD and plaques
- Amyloid-β oligomers (AβOs) initiate neurodegeneration



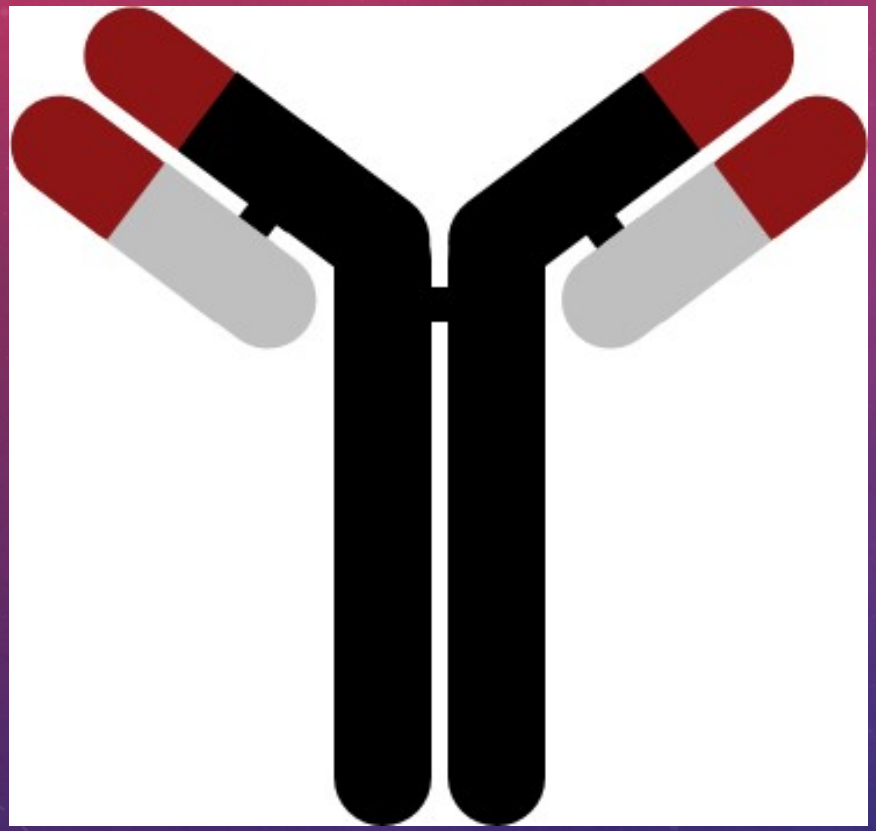
NIA 2016

BACKGROUND



Medical Care Corporation 2015

ANTIBODIES

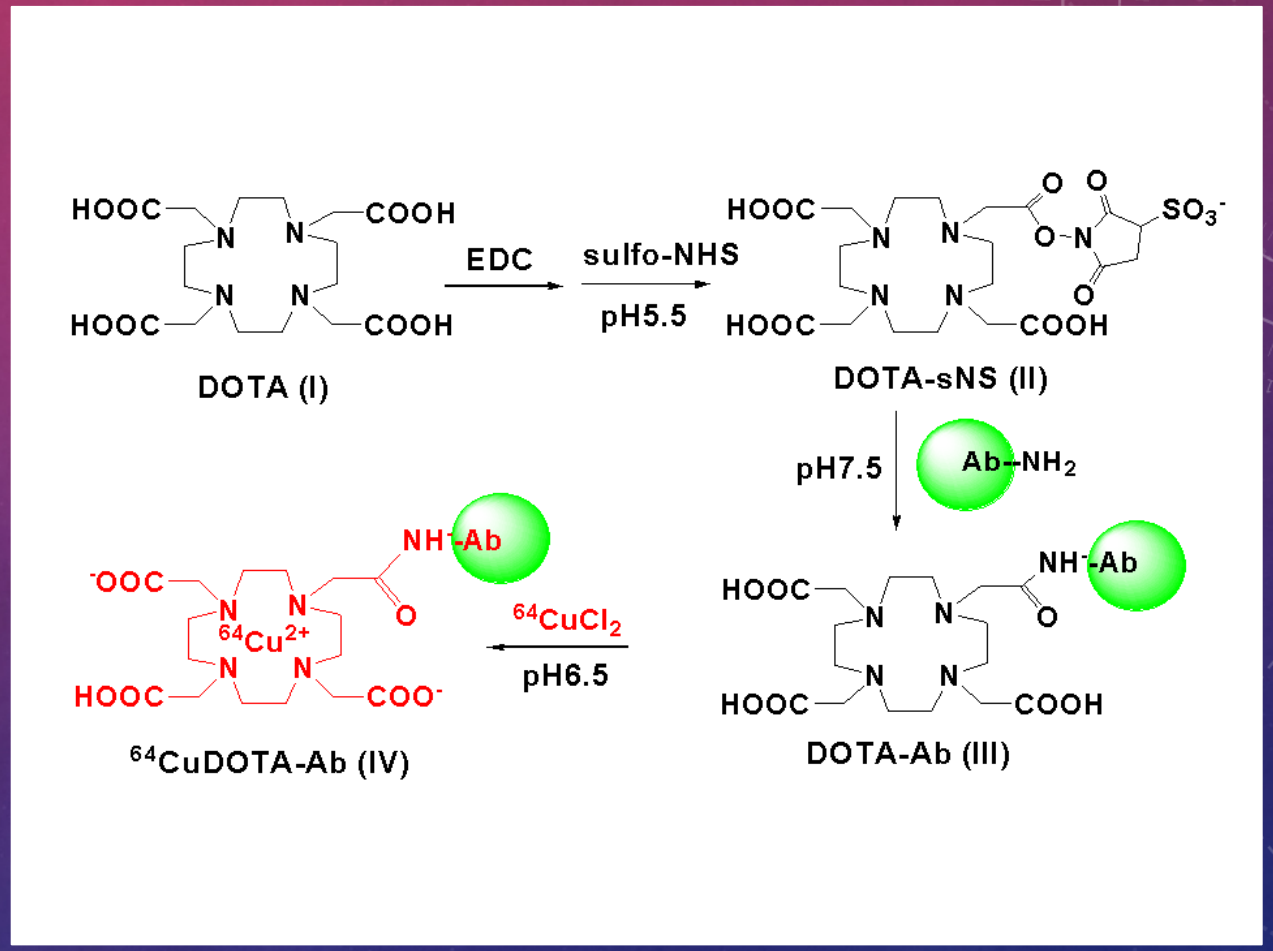


Cell Applications 2015

- Monoclonal
- NU4 - mouse
- ACU193 - human

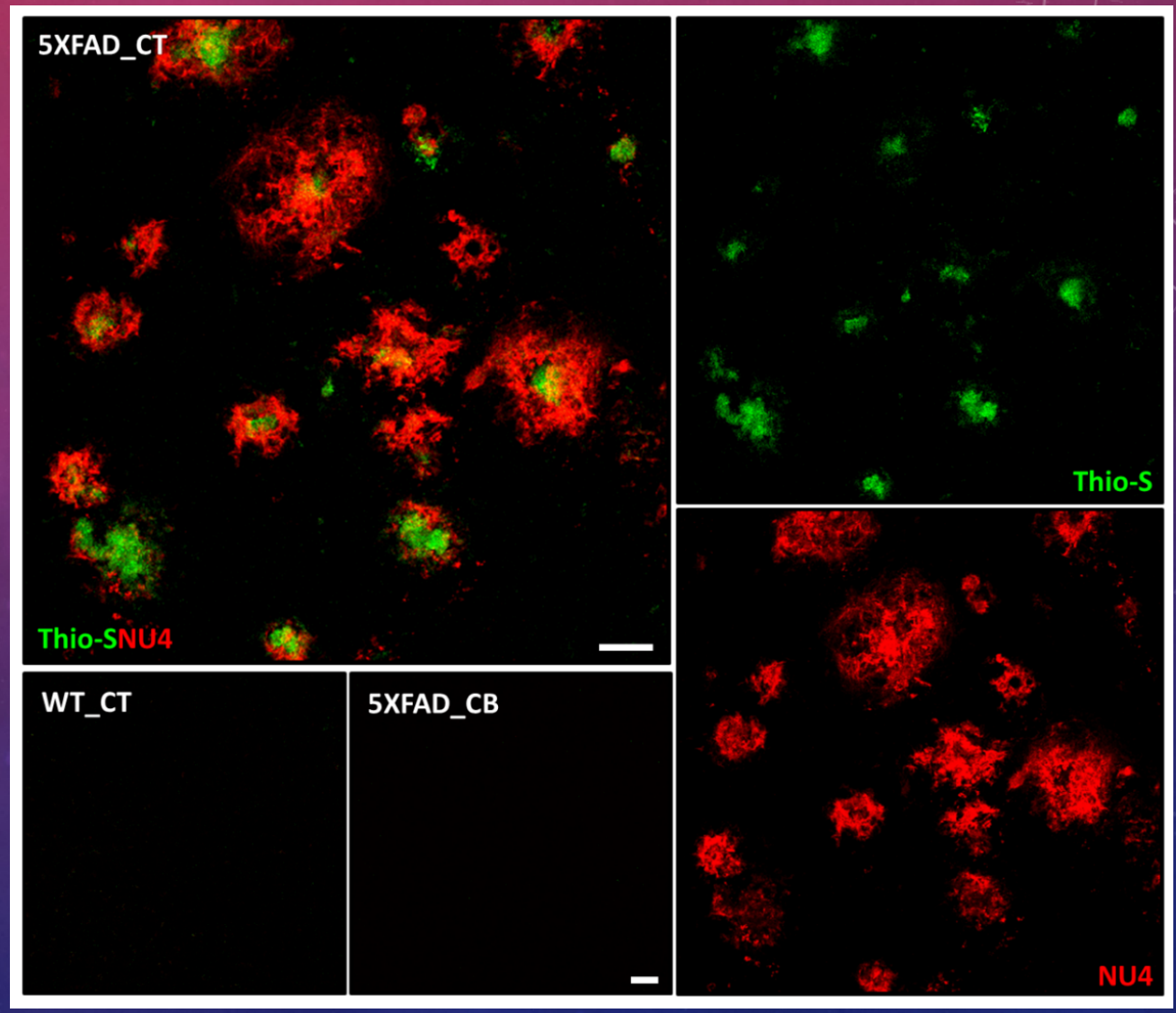
PET SYNTHESIS

- PET uses radioactive ligand
- Conjugation of DOTA cage to antibody
- Chelation with radioactive compound/element (^{64}Cu)



ABO ARE SEPARATE FROM PLAQUES

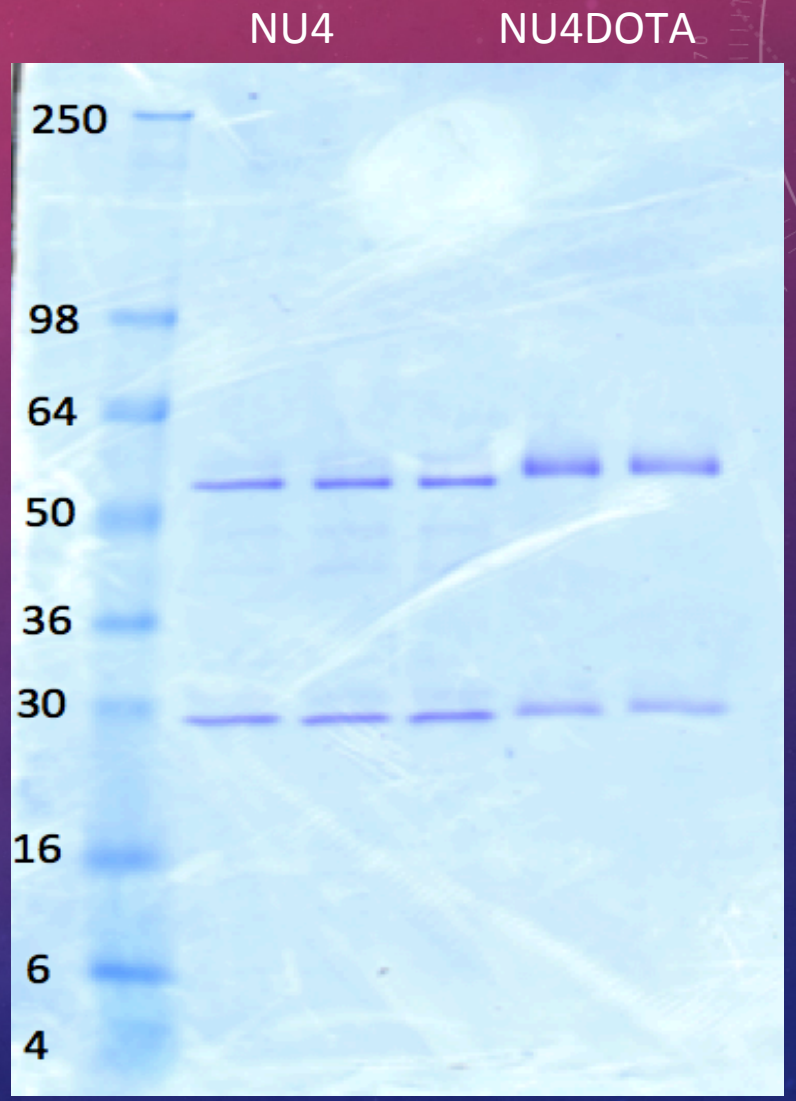
- Demonstrate that AβOs are separate from plaques
- Immunostained tg and wt mice with 568-NU4 (AβOs)
- Counterstained with ThioFlavin S (amyloid plaques)



Maira Bicca

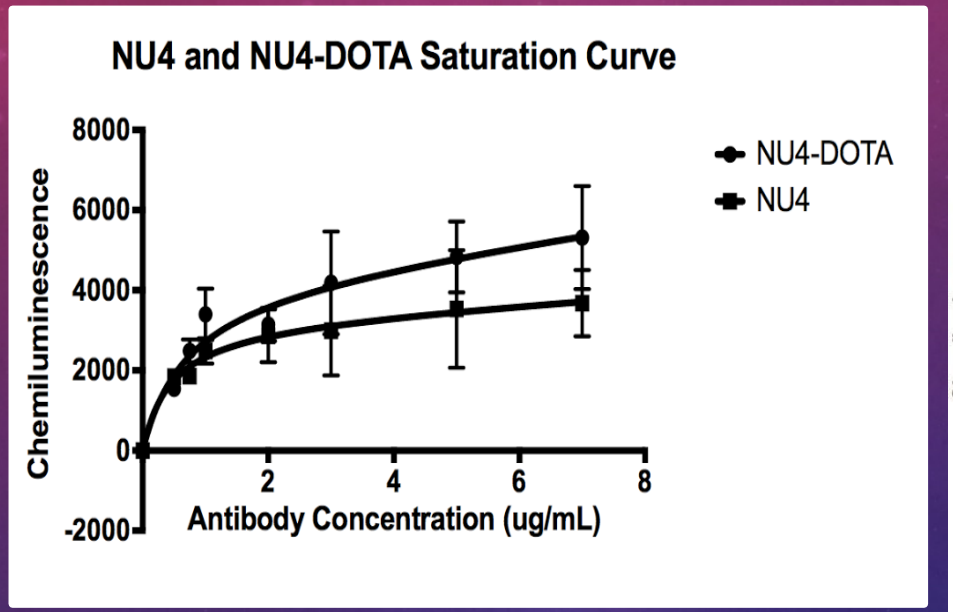
DOTA BINDS TO THE FC REGION OF NU4

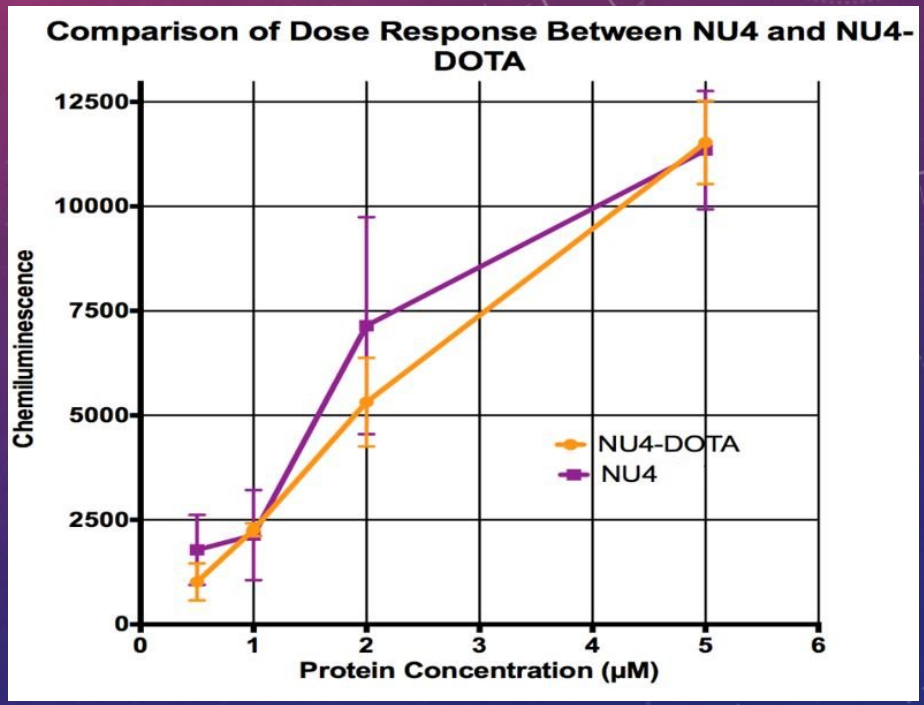
- Electrophoresis
- Confirm DOTA binds to constant region
- NU4: 54.091 kDa
- NU4-DOTA: 56.849 kDa
- 6-7 DOTA bound to heavy chain



NU4DOTA RETAINS IMMUNOREACTIVITY TO ABOS

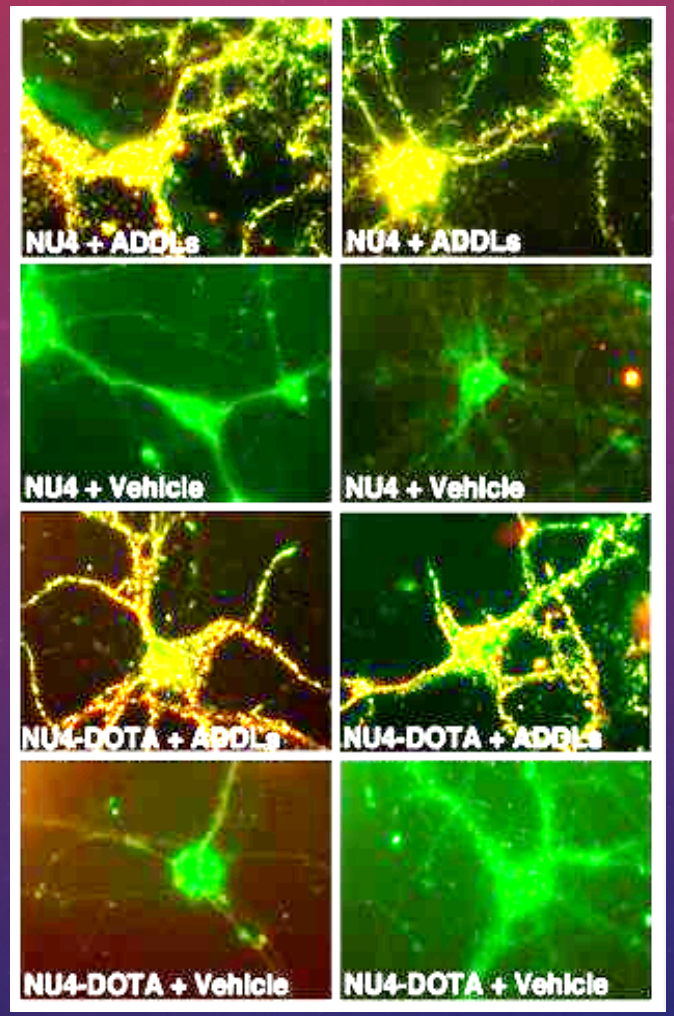
- Dot blots
- EC_{50}
- No decrease in immunoreactivity





NU4-DOTA COLOCALIZES WITH A**BOS** *IN VITRO*

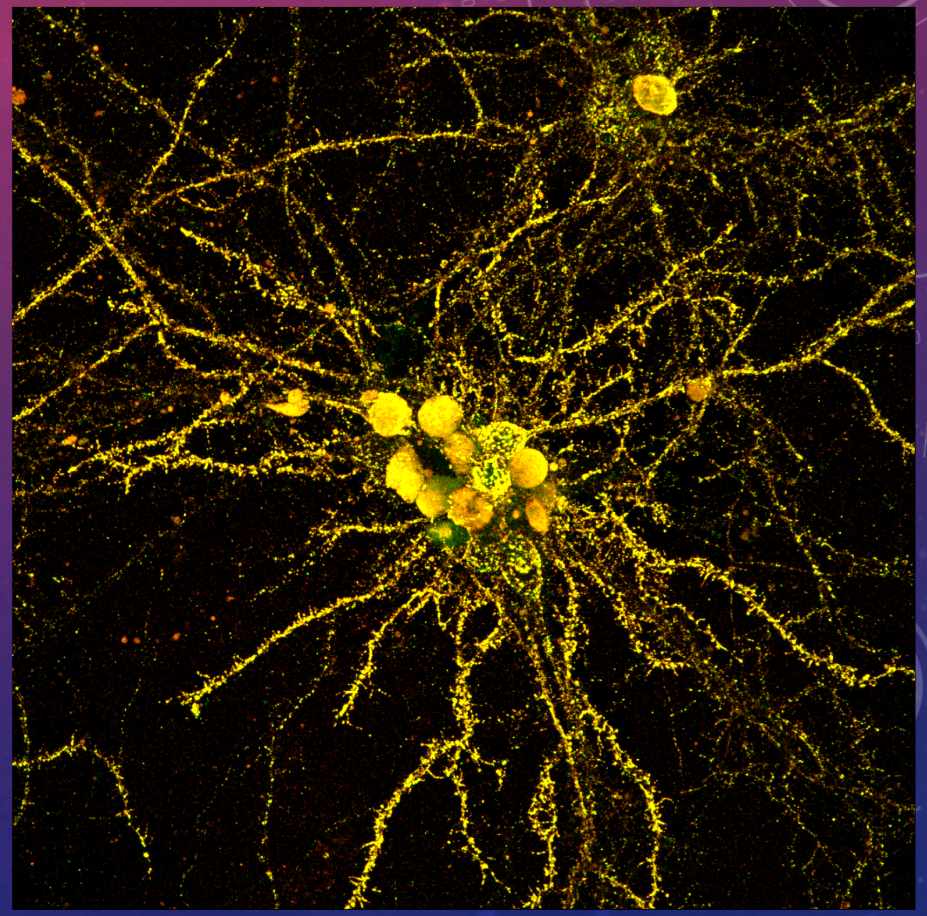
- NU4DOTA and FAM-AβOs in primary hippocampal neuron culture
- High levels of colocalization
- Retained immunoreactivity *in vitro*



Maira Bicca

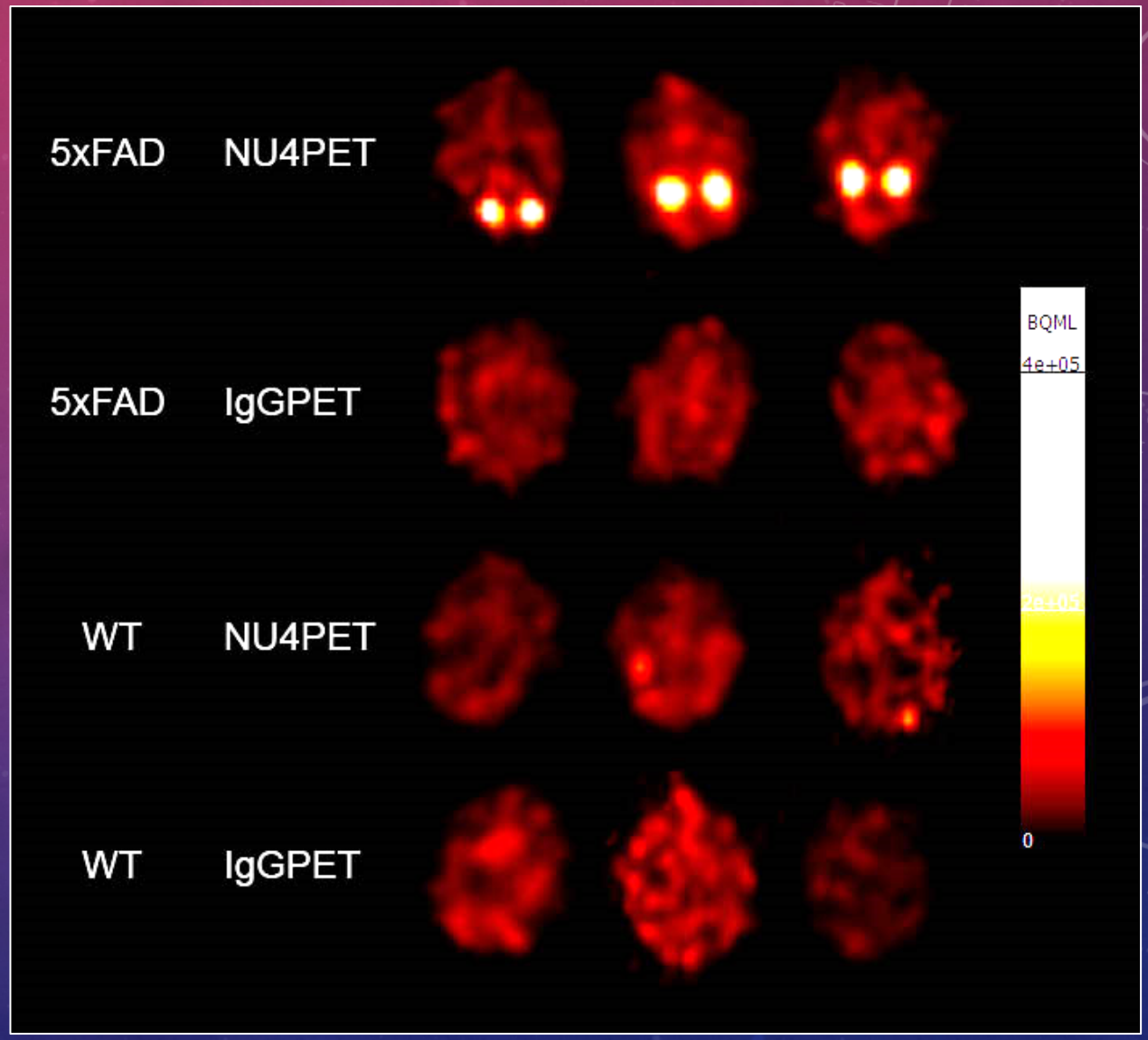
60x

FAM ADDLs/NU4



NU4PET DEMONSTRATE A STRONG AD DEPENDENT SIGNAL IN MICE

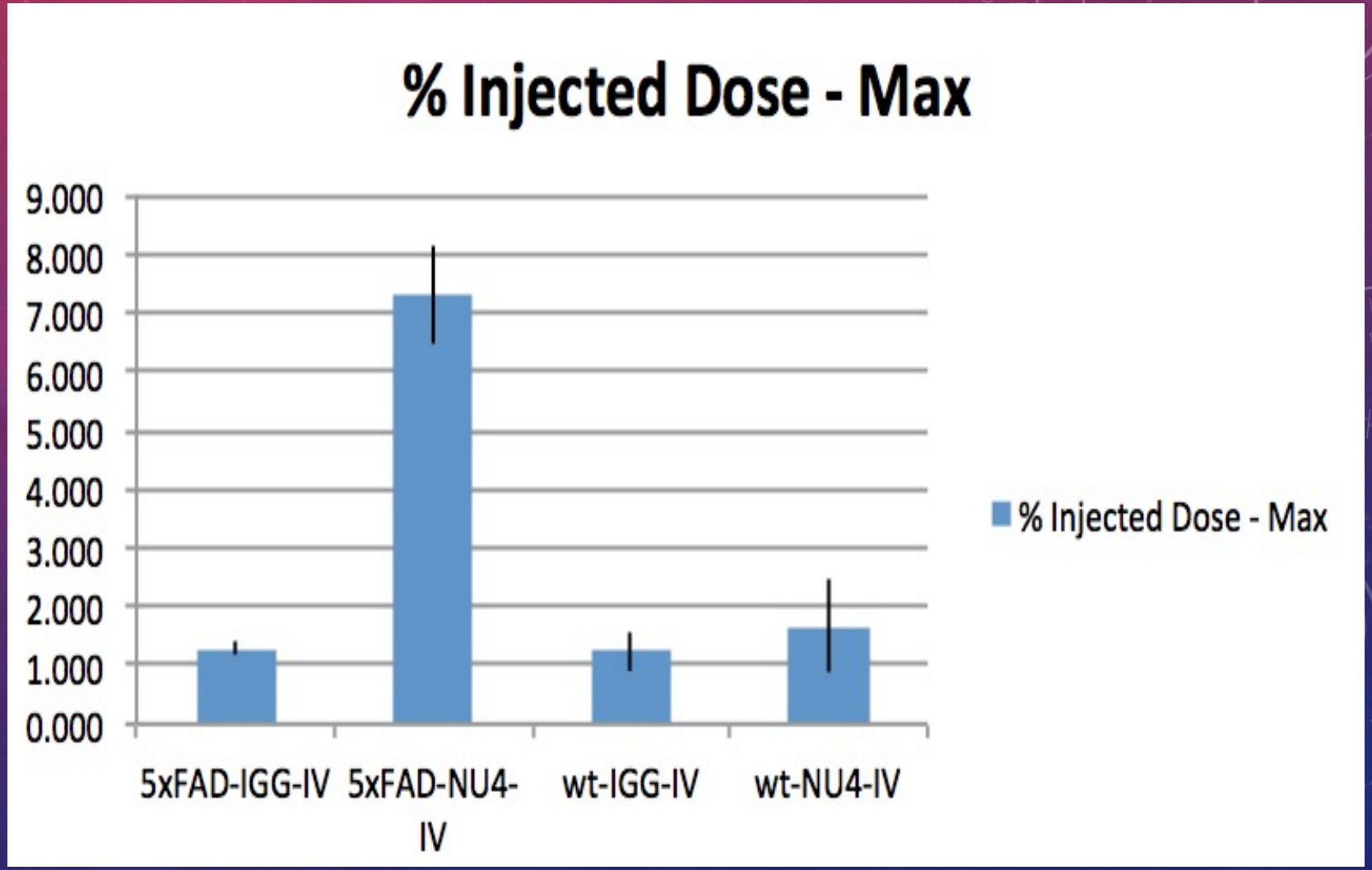
- Tg and Wt mice injected
- NU4PET
- IgGPET
- NU4PET demonstrates a strong AD dependent signal



Ting-Tung Chang

NU4PET DEMONSTRATES SUBSTANTIAL BRAIN UPTAKE

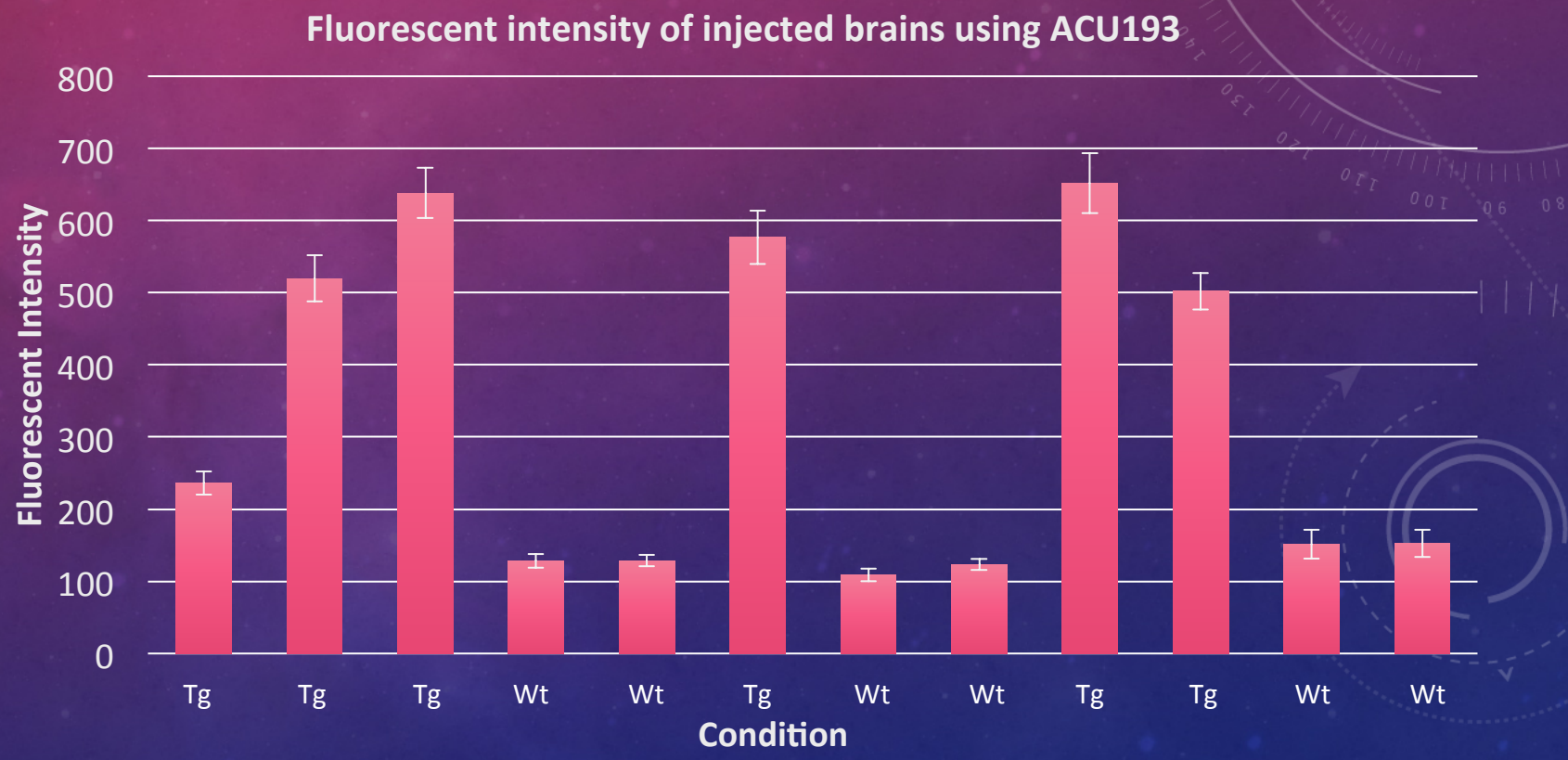
- Blood brain barrier is extremely difficult to cross
- Percent of injected dose retained similar to PiB and Florbetapir



Ting-Tung Chang

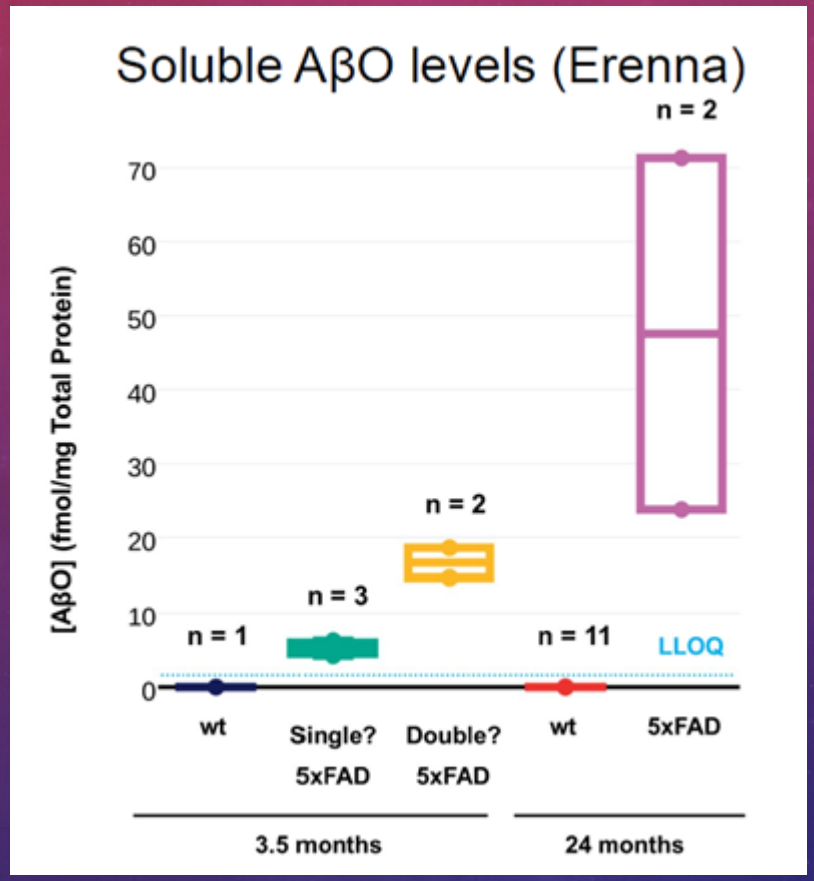
PET SIGNAL CORRELATES TO ACU193 IMMUNOFLUORESCENT INTENSITY

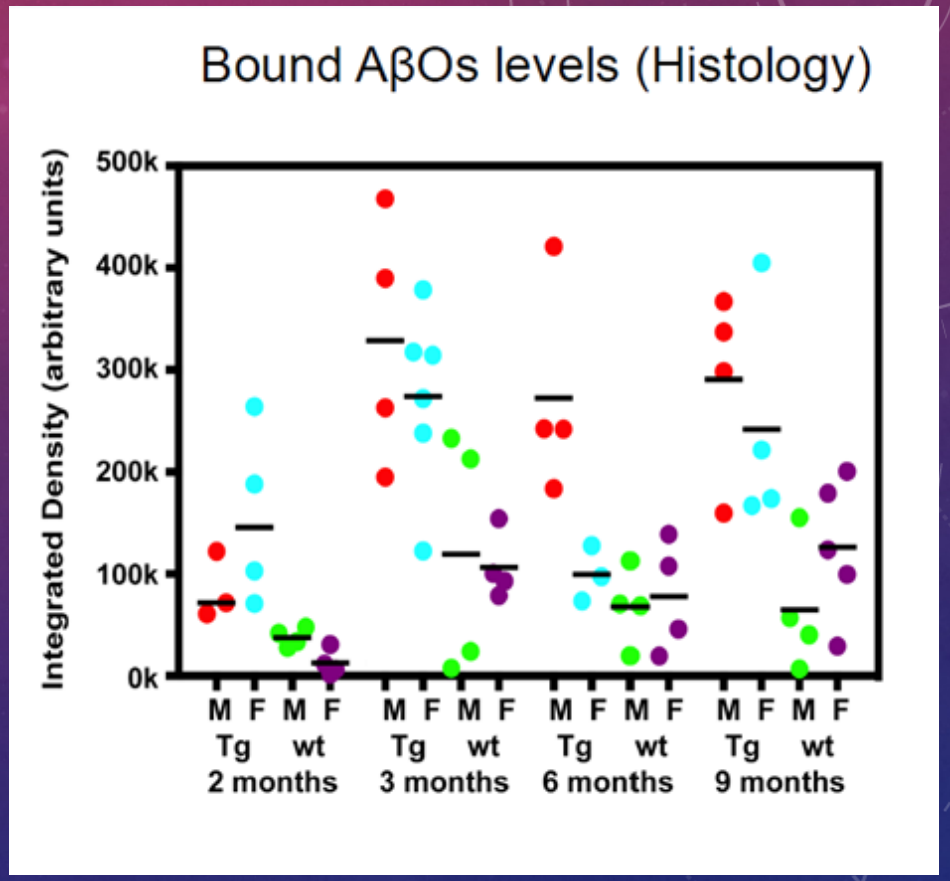
- Immunofluorescently labeled brains with ACU193
- Fluorescent intensity correlates to PET signal



ABOS ARE DETECTABLE AT 2 MONTHS IN 5XFAD MICE

- Target for diagnosis must appear before onset of symptoms
- ERENNA and histology
- AβOs are detectable at 2 months

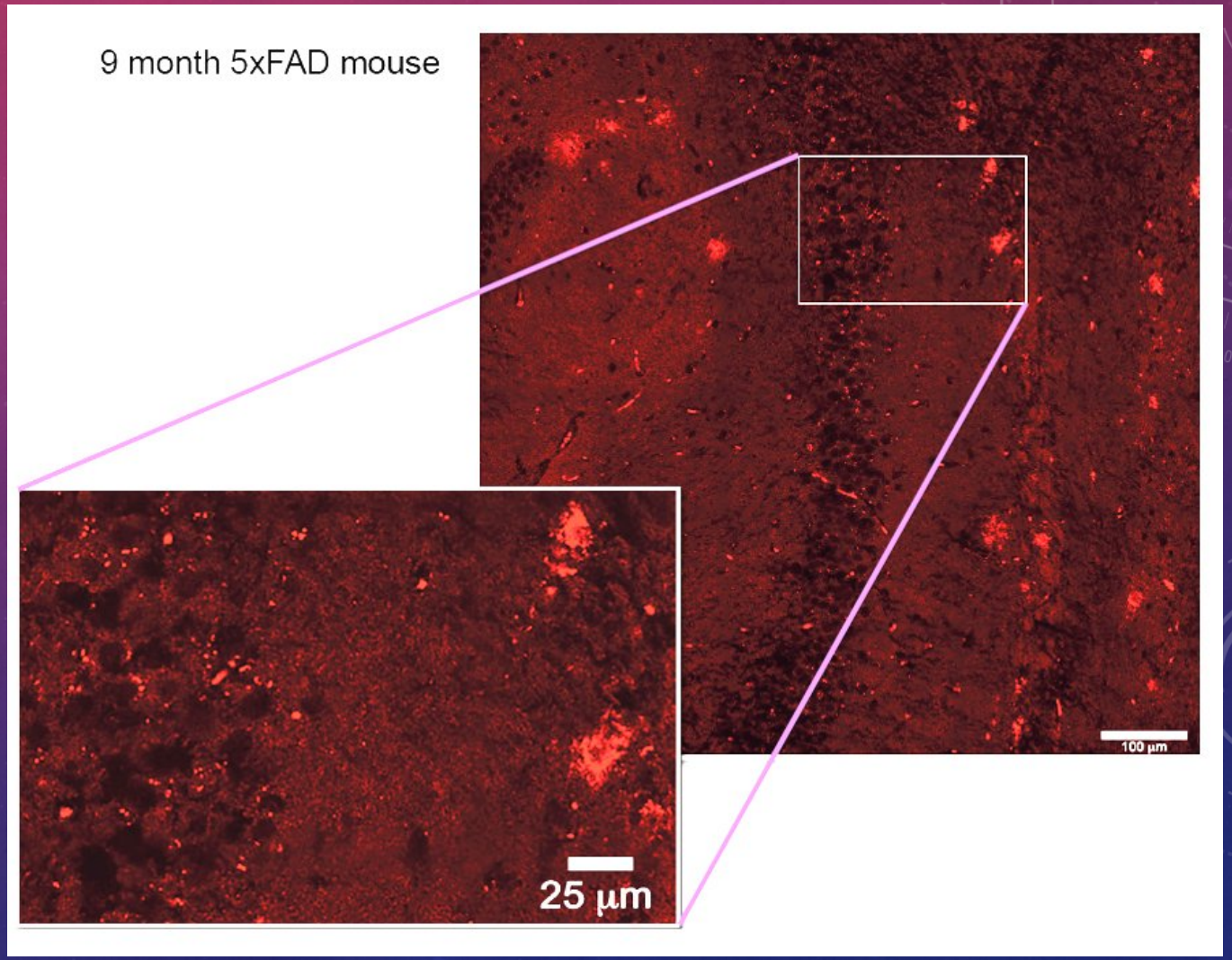




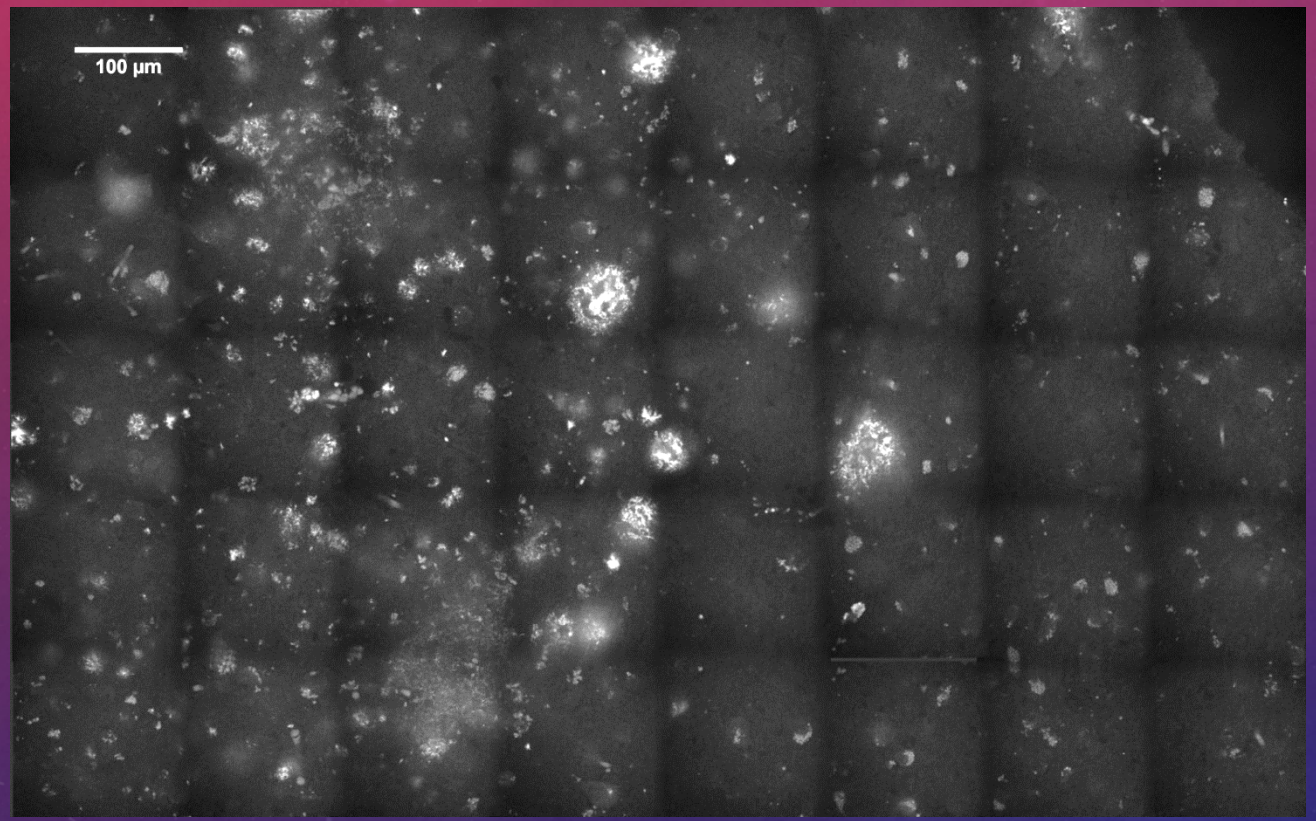
Erika Cline

NU4 DEMONSTRATES PLAQUE-LIKE AND PUNCTATE LABELING OF PYRAMIDAL LAYER IN 5XFAD MOUSE MODEL

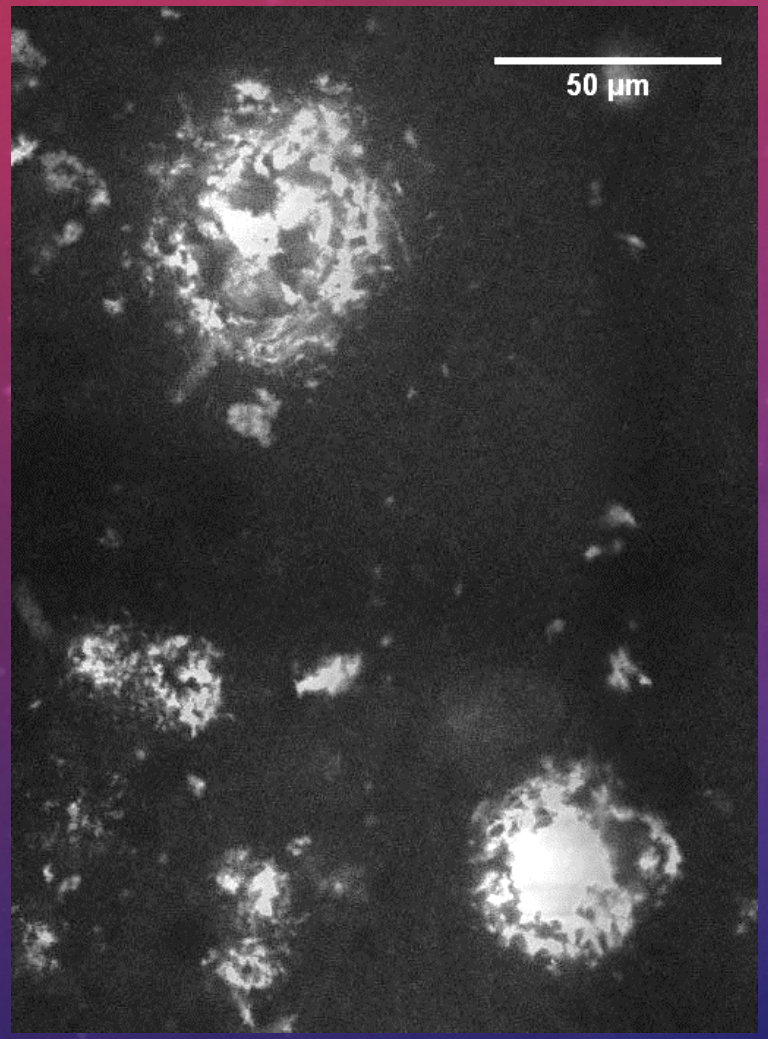
- Identify pathology seen in our 5xFAD mouse model
- Punctate and plaque-like labeling of the dendritic arbors
- AβOs found primarily in hippocampus, dentate gyrus and along the pyramidal layer

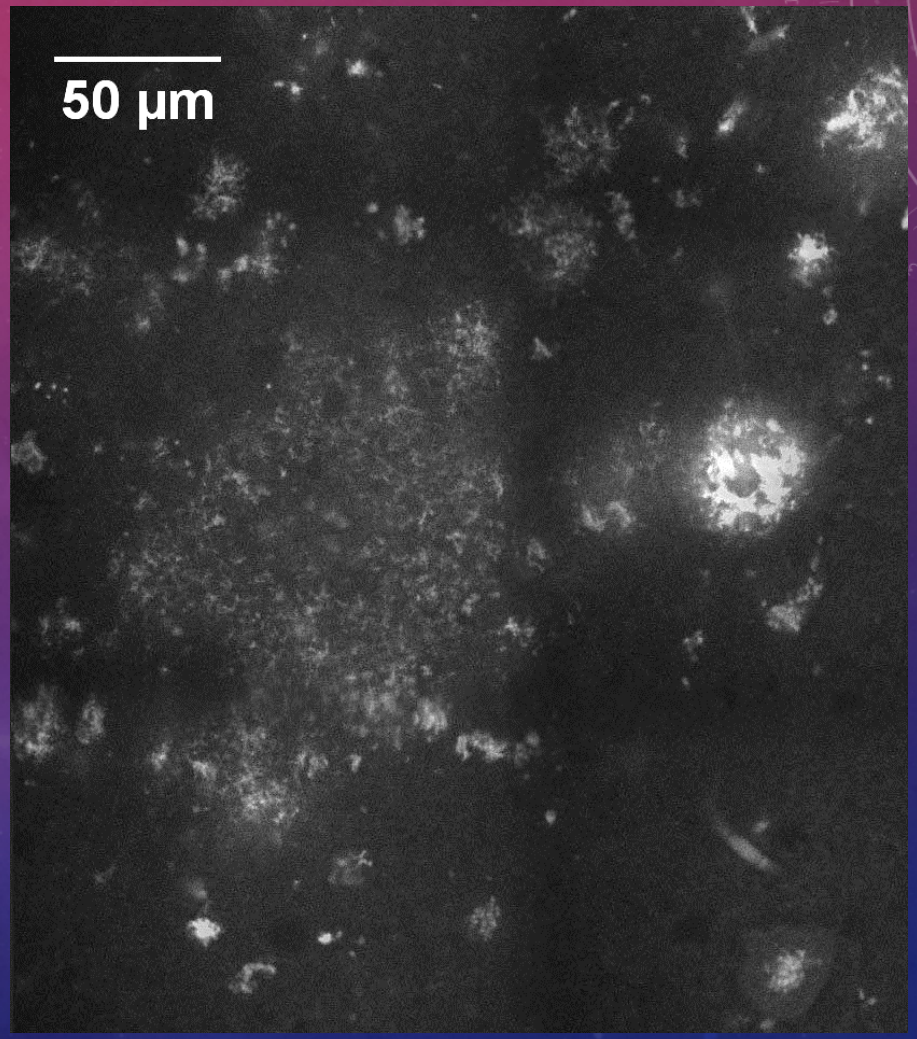


ACU193 DINSTINGUISHES BETWEEN DISEASED AND NONDISEASED HUMAN BRAINS



ACU193 SHOWS PLAQUE-LIKE, PUNCTATE, AND DIFFUSE PATHOLOGY IN HUMAN BRAIN TISSUE

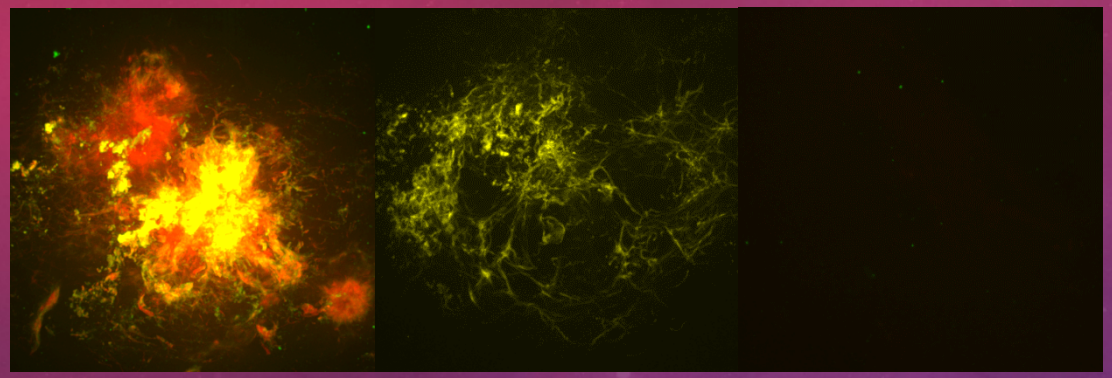




ACU193DOTA DISTINGUISHES BETWEEN 5XFAD AND WT TISSUES DOWN TO 2 MONTHS (NU4 AND ACU193DOTA)

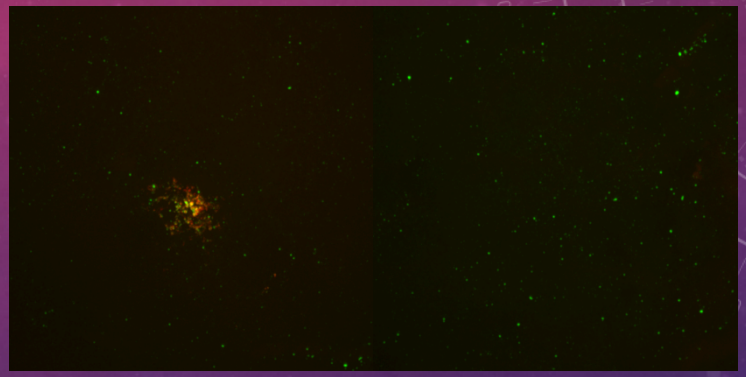
9M

Tg Tg Wt



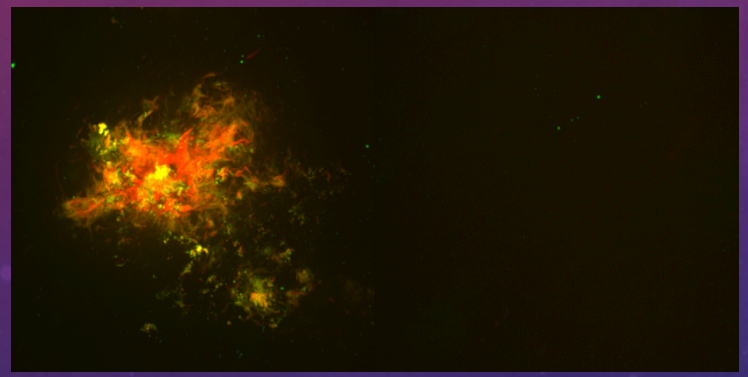
3M

Tg Wt



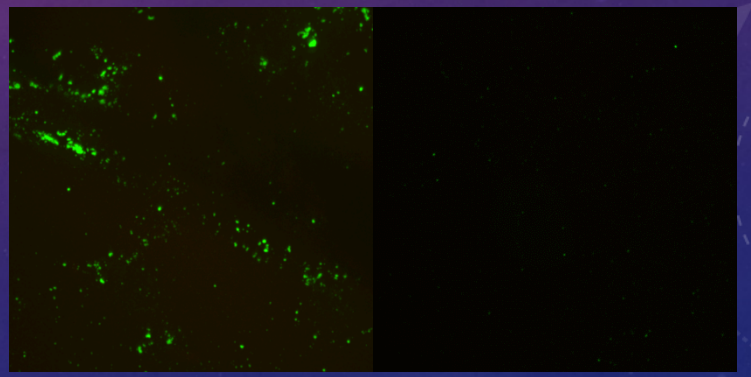
6M

Tg Wt

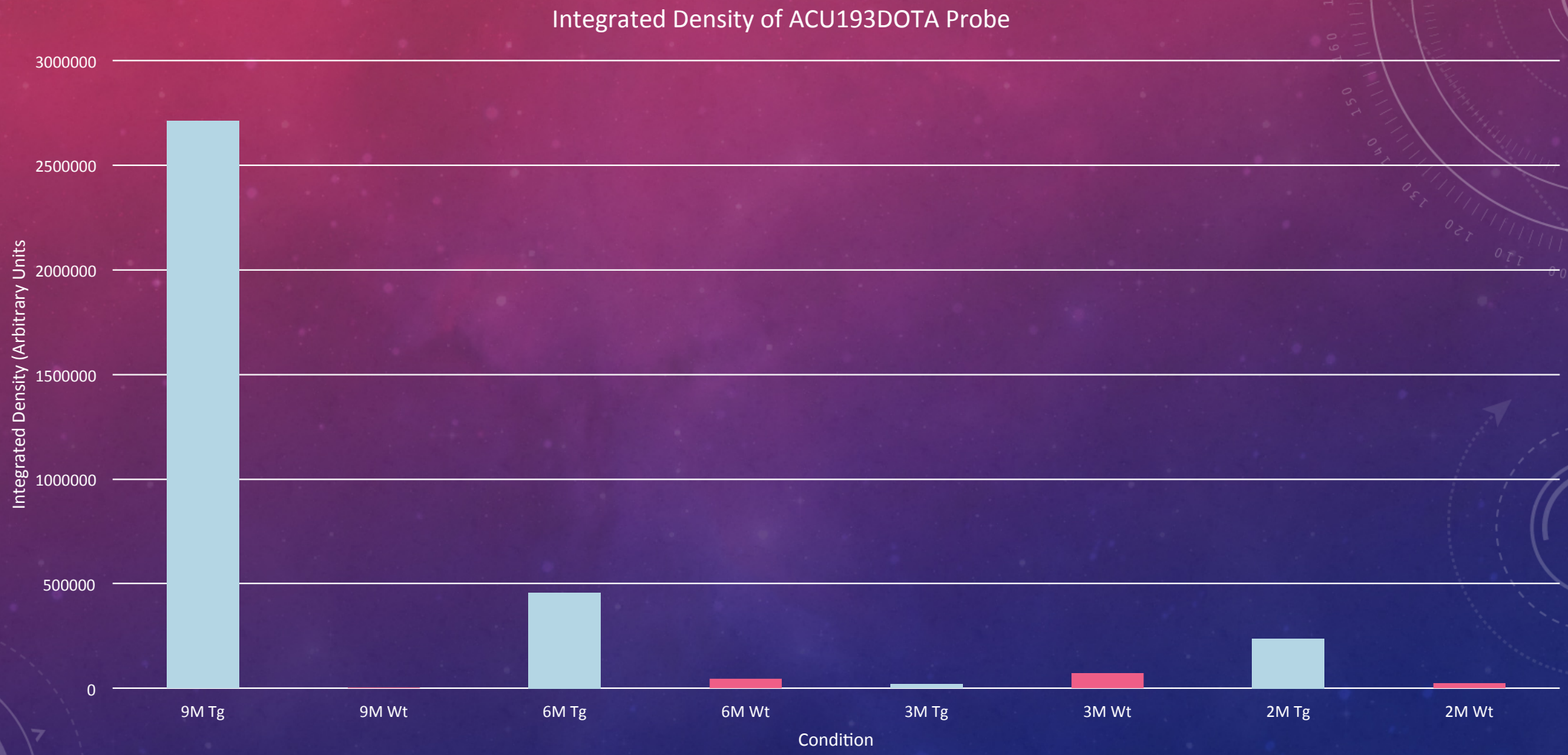


2M

Tg Wt



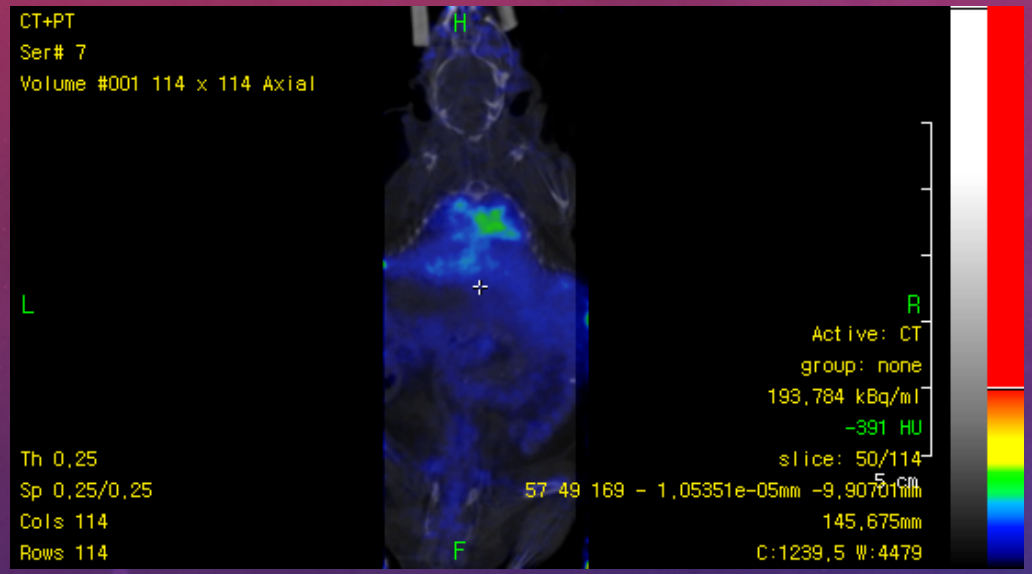
ACU193DOTA BINDS IN AN AGE DEPENDENT MANNER AND DISTINGUISHES BETWEEN TG AND WT MICE



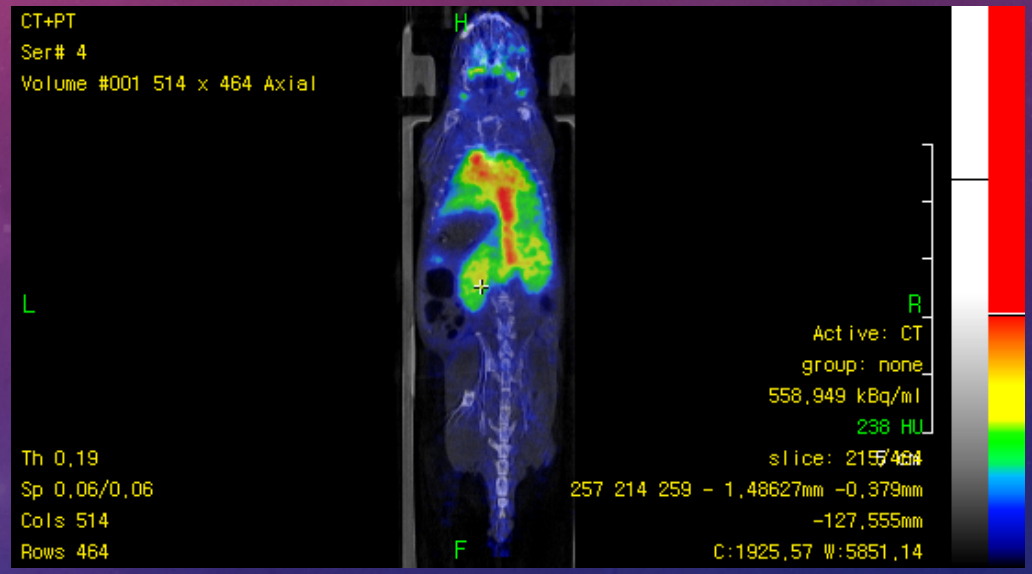
ACU193PET SHOWS PROMISE IN DISTINGUISHING BETWEEN DISEASED AND NONDISEASED MICE

DAY 1 RESULTS

Wild Type

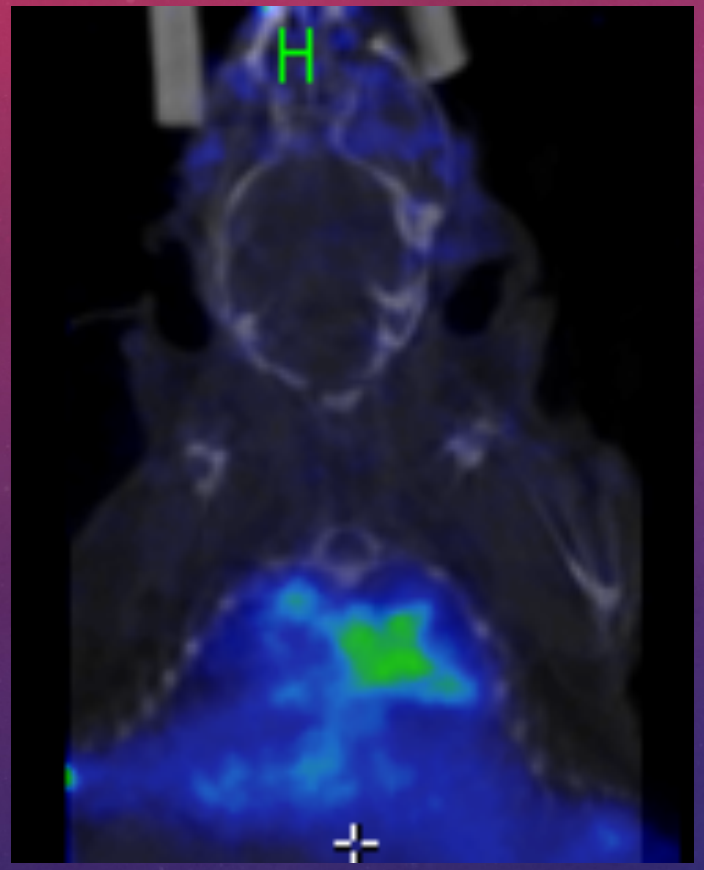


5xFAD

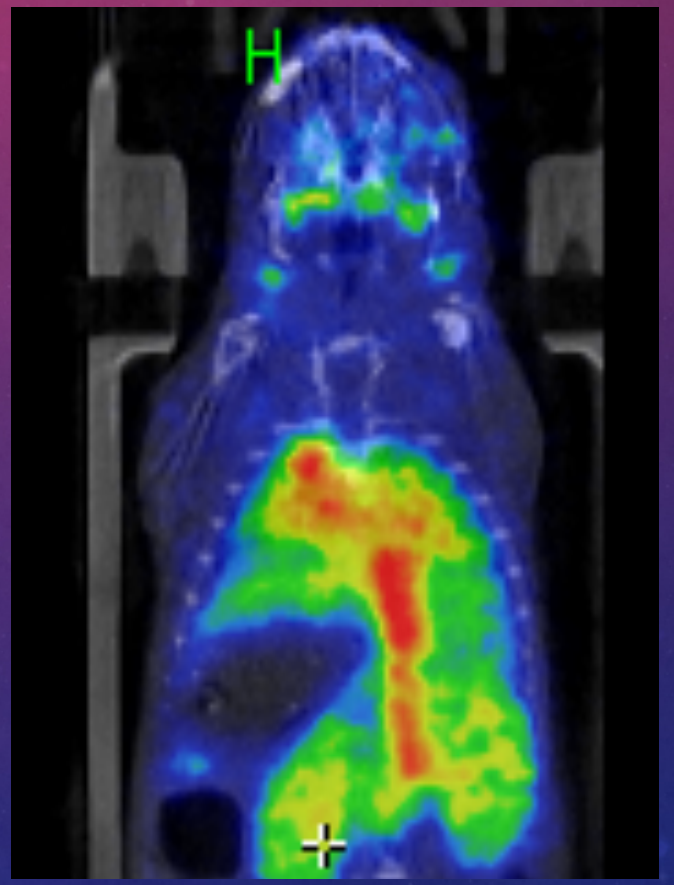


DAY 1 RESULTS

Wild Type



5xFAD



CONCLUSION

- ACU193PET demonstrates tremendous potential as an early diagnostic imaging tool for AD
- Future Work:
  - Substantiating AD specific probe signal by autoradiography using mouse and human brain sections
  - Dose curve PET to determine optimal dosing range in mid-stage AD (5xFAD model)
  - Evaluation of gross uptake and clearance in mice in vivo
  - Longitudinal analysis to determine earliest stages at which PET probe detects AβOs
  - Quantitative relationship between PET signals, AβOs detected histologically, AβOs detected biochemically, and memory loss
  - Human trial in 18 months
  - Dual MRI/PET

ACKNOWLEDGEMENTS

First and foremost I would like to thank Ms. Kirsten Viola for allowing me to work on this project with her and for being my mentor. I would like to thank Dr. William Klein for the opportunity to work in his lab and for all of the mentorship that he has also provided. I would also like to thank everyone who I have worked with at Klein Lab and all collaborators outside of Northwestern University for making this possible. Finally, would like to thank the SIR staff at IMSA and my parents for providing me with this opportunity and being a tremendous source of support.

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- NCI CCSG P30 CA060553 for Northwestern University's Center for Advanced Molecular Imaging

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